

AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of claims:

1-17. (Cancelled)

18. (Currently amended) A method of preparing a proliferation-regulated recombinant adenoviral vector ~~effectively~~, comprising the steps of:

preparing a proliferation-regulated ~~vector~~ plasmid by ~~(a)~~ preparing a restriction enzyme-recognizing unit in a ~~vector~~ plasmid ~~having a proliferation-regulating unit that includes, in order from upstream to downstream, and having~~ an E1A region, at least one protein-coding region in a E1B region or the entire E1B region, a poly(A) signal sequence, and a recombinase-recognizing sequence ~~in that order from upstream, by deleting~~ replacing both an endogenous promoter in the E1A region and an endogenous promoter regulating expression of the protein-coding gene ~~at least in the at least one protein-coding region in~~ in ~~[[of]]~~ the E1B region ~~and inserting with~~ restriction enzyme-recognizing sequences ~~respectively in these deficient sites, and (b) [[:]]~~

introducing a promoter expressing specifically in a target organ in the restriction enzyme-recognizing unit; and

~~additionally; integrating the proliferation-regulated vector plasmid into a vector plasmid having~~ containing an E1 region-deleted adenoviral genome ~~prepared by deleting the E1 region.~~

19. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector efficiently according to~~ claim 18, wherein the E1A region lacks a Rb protein-binding sequence.

20. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector efficiently according to~~ claim 19, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region and/or a 55KDa protein-coding region.

21. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector efficiently according to~~ claim 18, wherein each of the restriction enzyme-recognizing sequences ~~inserted to the sites lacking the endogenous promoter in the E1A region and the endogenous promoter regulating expression of the protein-coding gene at least in one protein-coding region of the E1B region has~~ include a blunt-end restriction enzyme site.

22. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector efficiently according to~~ claim 18, wherein the recombinase-recognizing sequence is LoxP, LoxH, or ~~[[the]]~~ a mutant sequence thereof.

23. (Currently amended) A method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene ~~efficiently~~, comprising the steps of: ~~(a) preparing a second therapeutic gene-expressing vector plasmid by allowing a recombinase to react with the proliferation-regulated vector plasmid by~~ (i) preparing a restriction enzyme-recognizing unit in a plasmid that includes, in order from upstream to downstream, an E1A region, at least one protein-coding region in a E1B region or the entire E1B region, a poly(A) signal sequence, and a recombinase-recognizing sequence, by replacing both an endogenous promoter in the E1A region and an endogenous promoter regulating expression of the protein-coding gene in the at least one protein-coding region in the E1B region with restriction enzyme-recognizing sequences, and (ii) introducing a promoter expressing specifically in a target organ in the restriction enzyme-recognizing unit; according to claim 18 ~~(b) preparing~~ [[and]] a first therapeutic gene-expressing vector plasmid ~~prepared by~~ (i) preparing a therapeutic gene-expressing unit by inserting in a plasmid in order from upstream to downstream a

recombinase-recognizing sequence and a restriction enzyme-recognizing sequence, (ii) inserting, in order from upstream to downstream, a constitutive high-expression promoter or a therapeutic gene-expressing promoter and a therapeutic gene in that order from upstream into the restriction enzyme-recognizing sequence of the vector plasmid containing a therapeutic gene-expressing unit, which is prepared by inserting a recombinase-recognizing sequence and a restriction enzyme-recognizing sequence respectively in that order from upstream; (c) preparing a second therapeutic gene-expressing plasmid by allowing a recombinase to react with the proliferation-regulated plasmid and the first therapeutic gene-expressing plasmid; [(,)] and additionally (d) integrating the second therapeutic gene-expressing vector plasmid into a vector plasmid having containing an E1 region-deleted adenoviral genome prepared by deleting the E1 region.

24. (Currently amended) The method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to claim 23, wherein the E1A region lacks a Rb protein-binding sequence.

25. (Currently amended) The method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to claim 24, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region and/or a 55KDa protein-coding region.

26. (Currently amended) A method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently, comprising the steps of: (a) preparing a proliferation-regulated adenoviral plasmid by (i) preparing a proliferation-regulated plasmid by preparing a restriction enzyme-recognizing unit in a plasmid that includes, in order from upstream to downstream, an E1A region, at least one protein-coding region in a E1B region or the entire E1B region, a poly(A) signal sequence, and a recombinase-recognizing sequence, by replacing both an endogenous promoter in the E1A region and an endogenous promoter regulating expression of the

protein-coding gene in the at least one protein-coding region in the E1B region with restriction enzyme-recognizing sequences, and introducing a promoter expressing specifically in a target organ in the restriction enzyme-recognizing unit, and (ii) integrating the proliferation-regulated plasmid into a plasmid containing an E1 region-deleted adenoviral genome; (b) preparing a therapeutic gene-expressing plasmid by (i) preparing a therapeutic gene-expressing unit by inserting in a plasmid in order from upstream to downstream a recombinase-recognizing sequence and a restriction enzyme-recognizing sequence, (ii) inserting, in order from upstream to downstream, a constitutive high-expression promoter or a therapeutic gene-expressing promoter and a therapeutic gene into the restriction enzyme-recognizing sequence of the therapeutic gene-expressing unit; and (c) allowing a recombinase to react with the proliferation-regulated adenoviral vector plasmid according to claim 18 and the [[first]] therapeutic gene-expressing vector plasmid prepared by inserting a constitutive high-expression promoter or a therapeutic gene-expressing promoter and a therapeutic gene in that order from upstream to the restriction enzyme-recognizing sequences of the vector plasmid having a therapeutic gene-expressing unit prepared by inserting a recombinase-recognizing sequence and a restriction enzyme-recognizing sequence respectively in that order from upstream.

27. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently~~ according to claim 26, wherein the E1A region lacks a Rb protein-binding sequence.

28. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently~~ according to claim 27, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region and/or a 55KDa protein-coding region.

29. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently;~~ according to claim 26, further comprising the steps of: mixing the proliferation-regulated

adenoviral ~~vector~~ plasmid and the first proliferation-regulated adenoviral ~~vector~~ plasmid, allowing a recombinase to react with the mixture, and then, transforming the ~~vetors~~ plasmids into each other.

30. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently,~~ according to claim 26, further comprising the steps of: cotransfecting the proliferation-regulated adenoviral ~~vector~~ plasmid and the ~~[[first]]~~ therapeutic gene-expressing ~~vector~~ plasmid into a recombinase-expressing cell.

31. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently~~ according to claim 30, wherein the recombinase-expressing cell is ~~a cell~~ prepared by making an adenoviral E1-region protein-expressing cell additionally express a recombinase.

32. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently~~ according to claim 23, wherein the recombinase-recognizing sequence in the ~~vector~~ plasmid containing a therapeutic gene-expressing unit is different from the recombinase-recognizing sequence in the ~~vector plasmid having that includes a proliferation-regulating regulated plasmid~~ plasmid ~~[[unit]]~~.

33. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently~~ according to claim 32, wherein the E1A region lacks a Rb protein-binding sequence.

34. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently~~

~~according to~~ claim 33, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region and/or a 55KDa protein-coding region.

35. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to~~ claim 23, wherein ~~[[the]] a drug tolerance gene in the vector plasmid having a proliferation-regulating regulated plasmid~~ ~~[[unit]] and [[the]] a drug tolerance gene in~~ ~~[[of]] the vector plasmid having a therapeutic gene-expressing unit~~ are different from each other, and Ori in the ~~vector plasmid containing a therapeutic gene-expressing unit~~ can duplicate pir genes such as R6K γ only in competent cell.

36. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to~~ claim 35, wherein the E1A region lacks a Rb protein-binding sequence.

37. (Currently amended) The method of ~~preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to~~ claim 36, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region and/or a 55KDa protein-coding region.

38-51. (Cancelled)